doi: 10.26114/pja.iung.154.2014.16.10

Polish Journal of Agronomy
2014, 16, 69–73

Evaluation of the selected forms of winter squash (*Cucurbita maxima* Duch.) for the content of free sugars and polysaccharides

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Abstract. The aim of the study was the evaluation of twelve forms of winter squash (*Cucurbita maxima* Duch.): cultivars Amazonka, Ambar and Karowita, 8 inbred lines (1235, 1236, 1243, 1245, 1249, 1307, 1309, 1310) and 1 F_1 hybrid (1311) for the content of free sugars and polysaccharides in their fruits. The entries were analyzed for dry matter, free sugars: fructose, glucose, mannose, saccharose; and polysaccharides: amylose, holocellulose, cellulose. Free sugars and amylose were analyzed by means of high performance liquid chromatography (HPLC), and holocellulose and cellulose using the gravimetric method. The hemicellulose content was calculated as the difference between the amounts of holocellulose and cellulose. The dry matter content in the fruit ranged between 4.0% and 25.1% of fresh weight, while the total sugars in the fruit ranged between 2.29% and 8.58%. The amylose content was estimated at 0.19% to 1.36% of fresh weight. The content of cellulose in the fruit ranged between 0.29% and 4.10%, while that of hemicelluloses – from 0.18% to 7.33% of fresh weight. Line 1310 and Ambar were characterized by the highest content of dry matter, amylose, holocellulose, cellulose and hemicelluloses in the fruit. A high additive correlation was determined between dry matter content in the fruit and the respective figures for holocellulose (r = 0.9175), cellulose (r = 0.9234), hemicelluloses (r = 0.8876), amylose (r = 0.7198) and the total carbohydrates (r = 0.8902).

key words: winter squash, polysaccharides, amylose, cellulose, hemicelluloses, HPLC.

INTRODUCTION

Winter squash (*Cucurbita maxima* Duch.) originating from North America has recently become more popular in Poland and Europe, due to its adaptability to the climatic conditions, stable yield as well as pro-health properties. Winter squash fruits are valuable and fit for direct consumption, but can also serve as a raw material for processing. Pumpkin fruits are rich in carotenoids, provide the highest contribution of beta carotene, showing strong antioxidative and anti-cancer qualities and a low content of alpha carotene, lutein, zeaxantin and violaxantin (Fraser and Bramley, 2004; Matus et al., 1993). Winter squash fruit flesh also contains a large amount of free sugars (glucose, fructose, saccharose) and polysaccharides (starch: amylose and amylopectin, cellulose, hemicelluloses) and provides a valuable source of dietary fibre. It is also rich in mineral salts such as potassium (K), phosphorus (P), magnesium (Mg), iron (Fe) and selenium (Se), while the seeds contain unsaturated fatty acids and some minerals, e.g. zinc (Zn). Additionally, pumpkin fruits contain vitamins C, E, B6, K, thiamine, riboflavin, and niacin.

With such a rich chemical composition, pumpkin offers a valuable component of a human diet, showing not only antioxidant and anti-cancer effects, but also hypoglycemic and anti-inflammatory properties.

Studies carried out by Xiong and Cao (2001) and Cai et al. (2003) confirmed that a pumpkin-rich-diet had a pharmacological effect on reducing blood glucose. Zhang and Yao (2002) demonstrated that water-extracted pumpkin polysaccharides possessed superior hypoglycemic properties. Li et al. (2005) concluded that the protein-bound polysaccharides from pumpkin fruit could be used as new agents in anti-diabetic treatment, because they could improve the tolerance of glucose, reduce the blood glucose level and increase the levels of serum tolerance of glucose (Li et al., 2005; Adams et al., 2011). According to Guillon and Champ (2000), patients with diabetes who consumed food rich in pectin, like pumpkin, showed a reduced need for insulin. The content of individual sugars in the flesh of pumpkin fruit and their ratios are important for human nutrition, in particular in the diet of patients, including the diabetics.

The aim of the study was the characterization of the twelve selected forms of winter squash (*Cucurbita maxima* Duch.) with regard to free sugars and polysaccharides in the fruits.

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Received 12 June 2013

MATERIALS AND METHODS

The twelve forms of winter squash, showing a diversified content of dry matter and carotenoid in the fruit, were used for the experiment (Sztangret et al., 2001). They included: 3 cultivars, bred in Dep. of Plant Genetics, Breeding and Biotechnology, Warsaw University of Life Sciences: Amazonka, Ambar and Karowita, 8 inbred lines (1235, 1236, 1243, 1245, 1249, 1307, 1309, 1310) and 1 F_1 hybrid (1311). The plants were grown in 2010 in the experimental field of Warsaw University of Life Sciences, Wolica. The experiment was laid out as a randomized block design, 6 plants of each entry per plot with spacing between plants 1.6 m x 1.2 m. The fruits were harvested at the end of September and stored at temp. 4–8°C till the end of November, when the samples were taken from randomly selected 4 fruits of every entry. The fruits were cut into small diagonal segments. Each segment was crushed and mixed, then 20 g samples were taken for dry matter analysis and 3.5 g ones were frozen at -20°C – for sugar and polysaccharide content analysis. In fresh samples a dry matter content was determined by drying the tissue at 104°C for 24 h in Hereaus (Thermo Scientific UT6060) dryer and the results were the mean values of 3 determinations for every fruit, expressed as a percentage of fresh weight. In frozen samples the analysis of free sugars: fructose, glucose, mannose and saccharose; and of polysaccharides: amylose, holocellulose, cellulose was carried out. The hemicellulose content was calculated as the difference between the content of holocellulose and cellulose. The results were also expressed as percentages of fresh weight. The method used for the analysis of free sugars and polysaccharides in winter squash fruits, described below, has been developed recently in Department of Wood Science and Wood Preservation, Faculty of Wood Technology, Warsaw University of Life Sciences (Radomski and Antczak, 2010).

Free sugars were extracted in cold water by the two freezing/unfreezing cycles. 1 g samples of tissue with 4 cm^3 of distilled water were frozen in glass tubes at -20°C, then after 2 hours they were unfrozen in a vessel filled with water and the extracts were collected in 10 cm^3 calibrated flasks. The procedure was repeated twice. The analysis of free sugars was made using the HPLC system (Shimadzu LC-20AD) equipped with DGU-20A degasser, CTO-20A oven, RID-10A refractometric detector and CBM-20A controller. The separation was conducted on Phenomenex Luna 5m NH_2 100A column at the mobile phase flow rate of 2.5 cm^3/min. The mobile phase composition was acetone: water mixture 90:10 (v/v). On the basis of standards (fructose, mannose, glucose and saccharose, POCH S.A. Company), free sugars were identified on the chromatograms and the areas of chromatographic peaks were calculated.

In order to remove the substances soluble in ethanol from the residue tissue after the extraction of free sugars, the samples were placed in glass tubes with 4 cm^3 ethanol and put to water bath at 60°C, then, every 30 min., ethanol was removed twice and replaced with fresh one. Subsequently, the tissue with 4 cm^3 distilled water was placed in glass tubes and dipped in water bath at 95°C. After 2 hours the extracts were collected to 25 cm^3 calibrated flasks. Three cycles of extraction were carried out. The analysis of amylose was made by the same HPLC system. 0.1 M $NaNO_3$ water solution was used as a mobile phase. The separation was conducted on a Phenomenex PolySep GFC Linear column at the mobile phase flow rate of 2.0 cm^3/min. Similarly as before, on the basis of the standard (amylose, Sigma-Aldrich Company), polysaccharide was identified on the chromatograms and the areas of chromatographic peaks were calculated.

For holocellulose and cellulose analysis the gravimetric method was applied. In the residue biomass the delignification process was carried out by supplementing 30 mg of crystal $NaClO_2$ and 4 cm^3 of water, placement of the sample in water bath at 70°C, after 10 min. adding 1 cm^3 acetic acid 1% and incubation for 3 hours. Then the residue was drained on Schott G3 funnels, swilled with distilled water, dried at 105°C overnight and weighed to make the holocellulose assessment. Subsequently, the sample was treated with 4 cm^3 of 1% NaOH at 95°C during 1 h, drained on Schott G3 funnels, swilled with distilled water, dried at 105°C overnight and weighed. In this way, the cellulose assessment was made. Hemicellulose was estimated as the difference between holocellulose and cellulose.

Statistic processing of the results was carried out using Statistica programme Version 10. Variation analysis was made and the Tukey's test was used in order to divide objects into homogeneous groups. Additionally, the regression analysis of the examined characteristics was applied.

RESULTS

The range of dry matter content in the fruit of the 12 *Cucurbita maxima* forms under study was between 4.0% and 25.1%, while the average value for all the entries was 12.4% (Table 1). Ambar cv. and inbred line 1310 were characterized by a significantly high dried matter content in the fruit (25.1% and 22.6%), while 1235 and 1309 lines showed the lowest level (4.0% and 4.7%).

The total sugars in the fruit flesh ranged between 2.29% (1235) and 8.58% (1310) and the average value for the 12 forms of pumpkin tested was 4.36% of fresh weight of fruit (Table 1). The fruits of 1310, 1311 and 1245 were characterized by the highest total level of sugars (8.58%, 6.88% and 5.58%, respectively), while 1235 and Karowita – by the lowest (2.29% and 2.48%, respectively).

Mannose showed on average the highest proportion of all the free sugars (1.24% of fresh weight of the fruit) (Table 1). The average amount of fructose was 1.13% of fresh weight, the level of saccharose – 1.00% of fresh weight and

Table 1. Characteristics of examined 12 forms of *Cucurbita maxima* with regard to the content of dry matter, glucose, fructose, mannose, saccharose and total sugars in the fruit.

Cultivar/line	Dry matter	Glucose	Fructose	Mannose	Saccharose	Total sugars
	% of fresh weight					
1235	4.0 a	0.78 a	1.20 abc	0.31 a	0.00 a	2.29 a
1236	7.0 a	0.25 a	0.85 ab	1.82 a	0.09 a	3.01 ab
1243	14.3 bc	0.66 a	1.13 ab	0.00 a	2.95 ab	4.74 abc
1245	16.2 b	0.80 a	1.07 ab	1.60 a	2.11 ab	5.58 abc
1249	7.2 a	1.33 ab	1.71 bc	0.52 a	0.04 a	3.59 ab
1307	8.5 ab	1.41 ab	1.20 abc	1.35 a	0.28 a	4.23 ab
1309	4.7 a	0.92 a	0.91 ab	1.61 a	0.00 a	3.44 ab
1310	22.6 e	1.52 ab	1.19 abc	1.38 a	4.49 b	8.58 c
1311	12.9 bcd	2.51 b	2.27 c	0.51 a	1.60 ab	6.88 bc
Amazonka	16.7 b	0.55 a	0.59 a	2.19 a	0.03 a	3.36 ab
Ambar	25.1 e	0.47 a	0.65 ab	2.69 a	0.31 a	4.13 ab
Karowita	9.3 bcd	0.62 a	0.75 ab	0.96 a	0.15 a	2.48 a
Average	12.4	0.99	1.13	1.24	1.00	4.36

a–e letters mean homogenous groups

that of glucose – 0.99% of fresh weight. The distribution of individual free sugars varied from entry to entry. For example, the saccharose had the highest share in the fruits of lines 1310, 1243 and 1245 (4.49%, 2.95% and 2.11%, respectively), while the highest proportion of glucose was noted in line 1311 (2.51%), that of fructose – in line 1311 (2.27%) and that of mannose – in Ambar, Amazonka and line 1236 (2.69%, 2.19% and 1.82%, respectively).

The starch-amylose content in the fruit of the twelve forms of winter squash ranged from 0.19% of fresh weight to 1.36% of fresh weight and the average level for all the treatments was 0.53% of fresh weight (Table 2). Line 1310 was characterized by statistically the highest amount of amylose in fresh fruit flesh (1.36%).

Holocellulose content in the fruit ranged from 0.47% of fresh weight (1309) to 11.43% of fresh weight (Ambar) with the average level for all the examined forms at 3.31% (Table 2). Ambar featured statistically the highest content of holocellulose in the fruit flesh (11.43%) constituting the first homogeneous group, while 1310 line gained the average of 8.23% of fresh weight and formed the second homogenous group.

The studied treatments attained the cellulose content between 0.29% and 4.10%, with the average of 1.60% (Table 2). Ambar and line 1310 were characterized by statistically the highest amount of cellulose in the fresh fruit tissue (4.10% and 3.95% of fresh weight, respectively) and constituted the first homogenous group. Similarly, the con-

Table 2. Characteristics of 12 forms of *Cucurbita maxima* in respect of the content of amylose, holocellulose, cellulose, hemicelluloses, cellulose share in holocellulose and total carbohydrates.

Cultivar/ line	Amylose	Holocellulose	Cellulose	Hemicelluloses	Cellulose contribution in holocellulose [%]	Total carbohydrates analysed [% of fresh weight]
	% of fresh weight					
1235	0.29 ab	0.82 a	0.54 a	0.29 a	65.0 h	3.40 a
1236	0.20 a	1.45 a	0.91 ab	0.54 ab	62.5 gh	4.66 ab
1243	0.78 bc	2.19 ab	1.17 ab	1.02 ab	53.1 cde	7.71 abc
1245	0.83 c	4.55 b	2.63 c	1.92 bc	57.9 efg	10.96 c
1249	0.20 a	1.00 a	0.77 a	0.23 a	77.0 i	4.79 ab
1307	0.29 ab	0.95 a	0.49 a	0.45 a	51.8 cd	5.47 ab
1309	0.44 abc	0.47 a	0.29 a	0.18 a	61.6 fgh	4.34 ab
1310	1.36 d	8.23 c	3.95 d	4.28 d	47.9 bc	18.17 d
1311	0.47 abc	2.33 ab	1.32 ab	1.01 ab	56.8 def	9.68 c
Amazonka	0.47 abc	4.66 b	1.99 bc	2.67 c	42.6 b	8.49 abc
Ambar	0.79 bc	11.43 d	4.10 d	7.33 e	35.9 a	16.34 d
Karowita	0.19 a	1.60 a	1.01 ab	0.59 ab	63.2 gh	4.27 a
Average	0.53	3.31	1.60	1.71	56.3	8.19

a–e letters mean homogenous groups

Table 3. Correlation coefficients for the traits tested.

Trait	Holocellulose	Cellulose	Hemicelluloses	Total sugars	Fructose	Mannose	Glucose	Saccharose	Amylose	Total carbohydrates
Dry matter	0.9175**	0.9234**	0.8876**	0.4826**	-0.1460	0.3253*	-0.0569	0.4115**	0.7198**	0.8902**
Holocellulose		0.9717**	0.9890**	0.3771**	-0.2311	0.3806**	-0.1052	0.2875*	0.6761**	0.8946**
Cellulose			0.9262**	0.4685**	-0.1791	0.3531*	-0.0621	0.3857**	0.7526**	0.9228**
Hemicelluloses				0.3091*	-0.2569	0.3869**	-0.1292	0.2179	0.6089**	0.8513**
Total sugars					0.4677**	0.1704	0.6382**	0.6776**	0.5573**	0.7486**
Fructose						-0.2622	0.8007**	0.1207	-0.0238	0.0545
Mannose							-0.1622	-0.4036**	0.0443	0.3365*
Glucose								0.2332	0.0774	0.2240
Saccharose									0.6223**	0.5473**
Amylose										0.7829**

** significant at level 0.01
* significant at level 0.05

tent of hemicelluloses in the forms of *Cucurbita maxima* ranged from 0.18% (1309) to 7.33% of fresh weight (Ambar) averaging 1.71% of fresh weight (Table 2). Ambar, for which the highest level of hemicelluloses in the fruit flesh (7.33%) was proved statistically, belonged to the first homogeneous group, and line 1310 with 4.28% of fresh weight belonged to the second homogeneous group. Line 1309 had the lowest content of cellulose and hemicelluloses in the fruit (Table 2).

The average proportion of cellulose in holocellulose of the fruit across all entries of winter squash was 56.3%, ranging from 35.9% (Ambar) to 77.0% (1249), while the average proportion of hemicelluloses in holocellulose was 43.7%, with the range between 23.0% (1249) and 64.1% (Ambar).

The total carbohydrates in the fruit ranged from 3.40% to 18.17% of fresh weight and the average for all the forms was 8.19% (Table 2), which was accompanied by 46% (Karowita) to 92% (1309) (with the average of 67.8%) of dry matter content in the fruit.

A highly significant additive correlation was proved between dry matter content in the fruit and holocellulose, cellulose, hemicelluloses, amylose, total sugars, saccharose and total carbohydrates in the fruit (Table 3). The highest values of correlation coefficients were obtained for the association between dry matter content and holocellulose ($r = 0.9175$), cellulose ($r = 0.9234$), hemicelluloses ($r = 0.8876$), total carbohydrates ($r = 0.8902$) and amylose ($r = 0.7198$).

DISCUSSION

For the 12 forms of winter squash examined in the experiment in 2010, a wide range of dry matter content in the fruit was determined (4.0% to 25.1% of fresh weight). Sztangret et al. (2001) found the range of dry matter content in the fruit of *Cucurbita maxima* cultivars to be as follows: 6.2%–15.4% in 1997, 5.7%–14.1% in 1998 and 6.55%–13.74% in 1999. In the study carried out in 2003–2004 (Seroczyńska et al., 2007) the dry mass content ranged from 4.97% to 15.97% in 2003 and from 6.01% to 23.95% in 2004. Simultaneously, the results confirmed that Ambar and inbred line 1310 were the forms with the highest amount of dry matter in the fruit in our germplasm collection. Cumarasamy et al. (2002), having studied six cultivars of winter squash grown in New Zealand, proved this trait to be at a level between 27.01% (Delica) and 33.19% (Ajihei), the values being much higher than those obtained in Poland.

Total sugars in the fruits of *Cucurbita maxima* forms under study ranged from 2.29% to 8.58% of fresh weight. Biesiada et al. (2011) reported the range of sugars content between 3.35% (Melonowa Żółta) and 6% (Amazonka), while Danilcenko et al. (2004) indicated a variability of this trait for 8 cultivars ranging from 2.23% (Bambino) to 12.24% (Stofuntowaja). The content of sugars in the fruits of winter squash depended on the genotype as well as postharvest conditions and time of sampling, as starch hydrolyzation during fruit storage affects the level of sugars.

In the research material the content of one fraction of starch – amylose – in the fruit ranged between 0.19% (Karowita) and 1.36% (1310) of fresh fruit tissue. According to Irving et al. (1999) the percentage of amylose in starch in the squash cv. Delica during fruit maturation, ripening and storage ranged between 20% and 25%, while the remaining 75–80% was amylopectine. In the experiment with eight cultivars originated from New Zealand Corrigan et al. (2001) confirmed the range of total starch content in the fruits from 0.49% (Bushfire) to 16.27% (Kaboten) at harvest and from 0.18% (Bushfire) to 7.31% (Kaboten) after 9–10 weeks' storage. The authors also noticed that the cultivars characterized by the highest amount of dry matter in the fruit had at the same time the highest level of starch in the fruit (Kurijman – 15.73%, Sweet Mama – 15.73%, Kaboten – 16.27%). The level of starch in the winter squash fruit reported in the cited publications depended on the genotype and time of storage before sampling, as during storage the starch present in winter squash fruits is degradated and converted into sugars. In our experiment, samples were taken 8 weeks after harvest, which may have reduced the amount of amylose.

The correlation coefficient for dry matter and amylose content in the fruit of *Cucurbita maxima* was 0.72, while Harvey et al. (1997) confirmed the correlation coefficient between a dry matter and total starch content in the fruit of winter squash at 0.83.

Ambar and inbred line 1310 had the highest amounts of holocellulose, cellulose and hemicelluloses in the fruit, while line 1309 gave the lowest results. It is worth pointing out that the share of cellulose in holocellulose proved very stable for each genotype and an inappreciable variability was shown (data not presented), which indicates a genotype-specificity of the components constituting cell walls of *Cucurbita maxima* fruit flesh.

Holocellulose, cellulose and hemicelluloses content in the fruit showed a strongly positive correlation with the amount of dry matter in the fruit ($r = 0.92$, $r = 0.92$ and $r = 0.89$, respectively). The strong correlation between polysaccharides and dry matter content in the fruits of winter squash could be a useful trait in breeding. The preliminary selection aimed at obtaining forms with a high amount of starch, cellulose or hemicelluloses in the fruit could be made on the basis of dry matter content, as the analysis is simple, quick and cheap.

The total of carbohydrates analysed accounted for about 46% (Karowita) to 92% (1309) of dry matter (on average – 67.8%). The residue of 8% to 54% consisted of amylopectin, lignin, and other undetermined components of the fruit.

CONCLUSIONS

1. The study showed a wide range of values for the characters studied in the twelve forms of *Cucurbita maxima.*
2. Line 1310 and Ambar cv. were characterized by the highest content of dry matter, amylose, holocellulose, cellulose and hemicelluloses in the fruit.
3. A high positive correlation was obtained for dry matter and amylose, holocellulose, cellulose and hemicelluloses content in the fruit of the research material, which can be useful in plant selection for breeding purposes.

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